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Sensitivity of the Generator of Spontaneous Motility in Chick Embryos to the Acute and Chronic Administration of MPTP

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Summary

The acute and chronic effect of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) on spontaneous motor activity and its development was studied in chick embryos. 1. From the 13th day of incubation, the acute effect of MPTP (30 mg/kg e.w., up to 60 min after administration) consisted in significant depression of spontaneous motility. From the 17th day, the effect of MPTP in supraspinal compartments of the CNS also began to participate in this depression. 2. The subacute effect of MPTP (up to 24 h after a single dose) was lethal for 11-day-old embryos. Conversely, in older embryos resting motility partly recovered, with signs of an inverse correlation to the embryo's age. The final effect, however, consisted in absolute failure of the hatching process. 3. The chronic effect of MPTP (3.57 mg/kg e.w./24 h, from the 4th to the 16th day of incubation) led to a developmental reduction of spontaneous motor activity, chiefly from the 8th to 12th day of incubation. 4. The interaction of nialamide (25 mg/kg e.w.), a blocker of monoaminooxidase produced disparate results with the effect of MPTP in young and old embryos.

Key words

Chick embryo – Spontaneous motility – MPTP – Nialamide – MAO

Introduction

Since its chance discovery in 1977, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has become very important in neurotoxicological research (for review see Markey and Schmuff 1986, Langston and Finnegan 1987, Kinemuchi *et al.* 1987, Kopin and Markey 1988). It has been evaluated as a potent nigrostriatal neurotoxin whose use leads to the development of a dopamine insufficiency syndrome. Damage to the nigrostriatal dopamine (DA) system has been found in man, various monkeys, dogs, cats, certain strains of mice and frogs. On the other hand, rats, guinea-pigs, rabbits and some mice seemed to be insensitive, even to repeated doses of MPTP (Schultz 1988). In addition to the species-related difference in the effect of MPTP, our attention was attracted chiefly by correlation to age, in two directions: 1. The phenomenon of age-related changes in the sensitivity of nigrostriatal DA neurones (Gupta *et al.* 1986, Kingston *et al.* 1987) and 2. The development of sensitivity to MPTP in the early stages of ontogenesis of the CNS (Mitilineau and Cohen 1984, Melaned *et al.* 1990). It was the latter relationship that first inspired our study of the effect of MPTP in developing chick embryos.

The second stimulus was the association between the effect of MPTP and monoaminooxidase (MAO) activity (Javitch *et al.* 1985, Johanessen *et al.* 1985) and the structural similarity between MPTP and haloperidol, which is able to induce extrapyramidal symptoms (Goodman and Gillman 1980). Both these aspects have already been verified in the development of the spontaneous motility of chick embryos (Sedláček 1970, Sedláček and Corner 1980).

The above circumstances provided sufficient grounds for undertaking a study of the development of the acute and chronic effects of MPTP and their association with MAO activity.

Material and Methods

The experiments were carried out on White Leghorn embryos incubated under the usual standard conditions. The test substances in water solutions were administered acutely from the 11th to the 19th day of incubation. They were sprayed onto the egg membrane over the egg air space – MPTP in a concentration of 30 mg/kg e.w. (25 μl) and nialamide (NAD) in a concentration of 25 mg/kg e.w. (50 μl).

Spontaneous motor activity was recorded in the intact eggs by the vibration technique, 20 min before and 60 min after administering the test solution

(Sedláček 1977), at the intervals given in the individual experimental series.

The chronic administration of MPTP (in a mean concentration of 3.57 mg/kg e.w./24 h) was ensured by continuous withdrawing the solution by suction from the 4th to the 8th day of incubation (series A), from the 4th to 12th day (series C), from the 4th to the 16th day (series E) and from the 8th to the 12th day (series 8-12). Spontaneous motor activity was always recorded for 60 min on the 17th day of incubation.

Spinal embryos were obtained by decapitation on the 2nd day of incubation (Sedláček and Doskočil 1978).

Each of the numerical data in this study is based on the results from at least 10 embryos.

Results

The first part of the results comprises the effects of MPTP after acute administration. From the 13th day, in a dose of 30 mg/kg e.w., MPTP caused significant depression of spontaneous motility, which in 13- and 15-day-old embryos was complete throughout. In older embryos two phases could be distinguished in this depression.

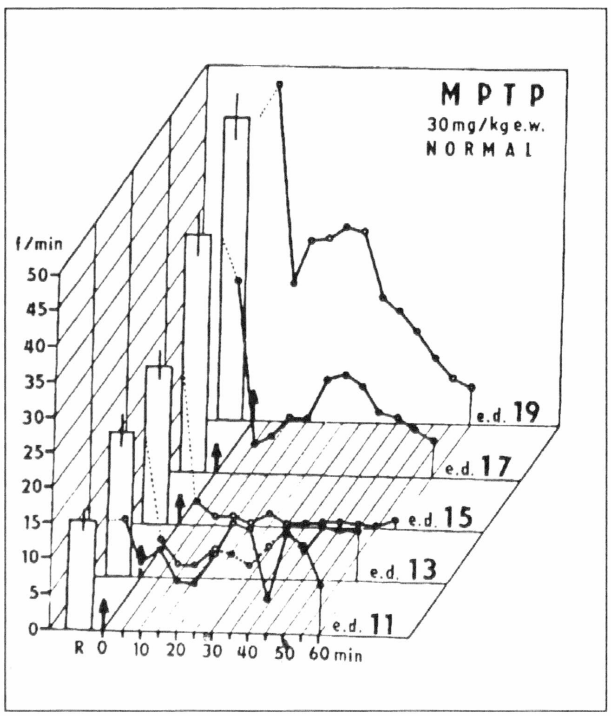

Fig. 1
Development of the acute effect of MPTP on the spontaneous motility of chick embryos. x axis: R- 20 min resting motor activity (M ± S.E.M.); time in minutes after administering MPTP (arrow). y axis: spontaneous movement frequency per min. z axis: embryos' age in days of incubation (n = 10 per day).

The first one was an abrupt decrease in the frequency of spontaneous movements during the first 5 min after administering MPTP, with a partial tendency to recovery of the original resting activity during the first half hour. The second phase was more gradual and had evidently not terminated at the end of the first hour after administering MPTP (Fig. 1).

A comparison of this result in normal embryos with the acute effect of MPTP in spinal embryos (Fig. 2) is significant, especially on the 17th day

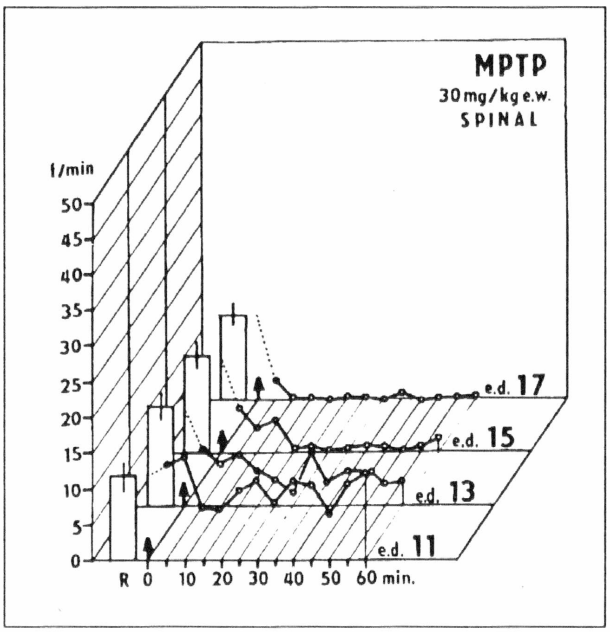

Fig. 2
Effect of MPTP on the spontaneous motility of spinal chick embryos. Details as in Fig. 1.

of incubation. The result in 11-, 13- and 15-day-old spinal embryos did not differ significantly from the results in 17-day-old spinal preparations, in which the second phase of the reaction did not appear at all and spontaneous motility behaved exactly in the same way as in 15-day-old chick embryos.

A single dose of MPTP, however, had further consequences as well as mere changes in motility during the first 60 min after administration. From the 13th day, depression persisted for over 6 h after its administration. Significant developmental differences appeared after 24 h (Fig. 3) and in 11-day-old embryos, in which the initial motility changes were nonsignificant, the effect of MPTP during the first 24 h was lethal without any exception.

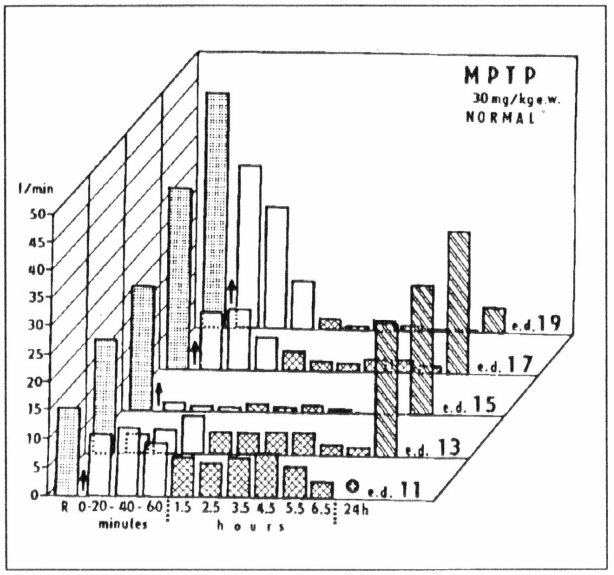

Fig. 3
Development of the effect of MPTP in chick embryos during the first 24 h after a single dose. x axis: resting motility (see Fig. 1); time in minutes and hours after a single dose of MPTP (arrow). *y* axis: spontaneous movement frequency per min. *z* axis: embryos' age in days of incubation up to the instant of administering MPTP. + = lethal effect of MPTP.

In older embryos, the behaviour of spontaneous motility during the first 24 h recovered best in 13-day-old embryos and worst in 19-day-old embryos. In 13- to 17-day-old embryos spontaneous motility recovered to the same values although, of course, with different initial resting values and even compared with the corresponding values in the control embryos 24 h older (Tab. 1). In 13- and 15-day-old embryos, spontaneous motility completely recovered or even increased in 24 h, whereas in 17-day, and particularly in 19-day-old embryos recovery was – more or less – only partial.

The final consequence of single (acute) administration was seen at the end of incubation. Although all the embryos given a single dose of MPTP survived up to the end of incubation, none of them hatched out and they all died on the 21st day of incubation.

In another series of experiments, the effect of MPTP (30 mg/kg e.w.) was combined with the simultaneous or successive effect of nialamide (25 mg/kg e.w.), using 13- and 17-day-old embryos. Successive administration followed the patterns MPTP– >NAD and NAD– >MPTP, in each case with a 15-min interval. The second combination allowed a rough evaluation of the activity of plain nialamide in a dose of 25 mg/kg e.w. during the first 15 min after its administration: in 13-day-old embryos the effect consisted in nonsignificant fluctuation of the frequency of spontaneous movements and in 17-day-old embryos

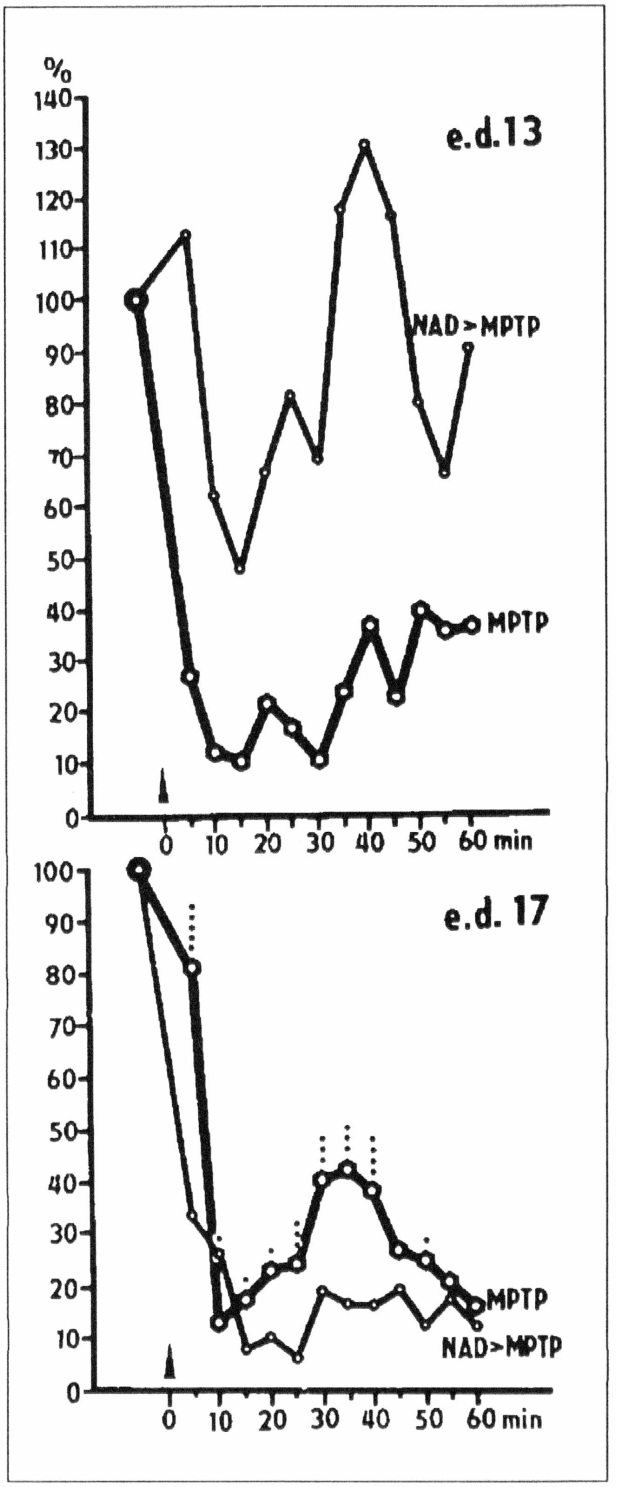

Fig. 4
The effect of MPTP and the combination NAD– >MPTP on spontaneous motility in 13- and 17-day-old chick embryos. Abscissa: R-resting motility, time in minutes after administering MPTP. Ordinate: percentage of resting activity before administering the tested drugs. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 1

Recovery of spontaneous motility after a single dose of MPTP

Motility before administering MPTP		Motility 24 h after administering MPTP		Motility in the control		
Age in days of incubation	f/min[x]	f/min		f/min of incubation		Age in days
13	16.4 ± 2.2	N.S.[xx]	23.9 ± 3.9	N.S.	18.9 ± 2.0	14
15	21.4 ± 1.8	*	30.9 ± 3.2	N.S.	35.4 ± 1.9	16
17	21.4 ± 2.1	*	25.8 ± 3.0	**	37.5 ± 2.5	18
19	43.4 ± 2.6	**	4.5 ± 1.3	***	44.2 ± 3.1	20

* spontaneous movement frequency per min (M ± S.E.M.)

[xx] N.S. – nonsignificant difference between the adjacent values, statistical significance * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$

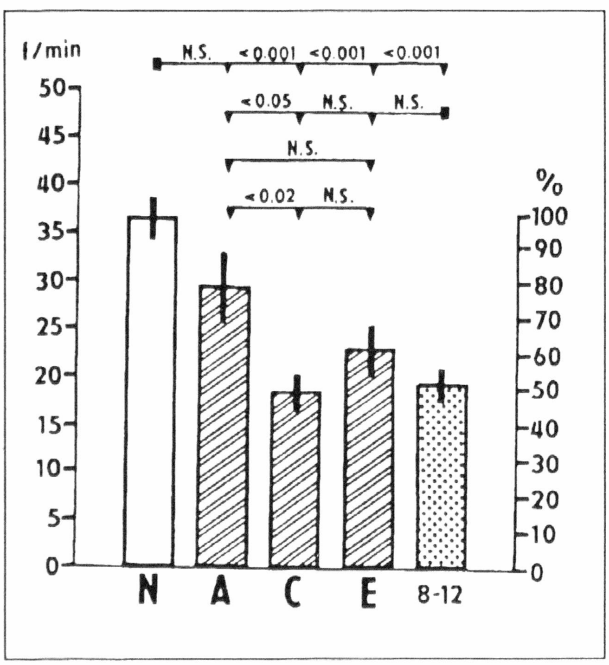

Fig. 5

Consequences of the chronic continuous administration of MPTP for the development of spontaneous motility in chick embryos and the result on the 17th day of incubation. Abscissa: normal embryos; A- MPTP administered from the 4th to the 8th day of incubation, C- from the 4th to the 12th day, E- from the 4th to the 16th day; (8-12) – MPTP administered from the 8th to 12th day of incubation. Ordinate: left – spontaneous movement frequency per min in 17-day-old embryos (M ± S.E.M., n = 10); right - magnitude of spontaneous motility expressed as a percentage of the control value (N).

at the onset of a depressive effect 10-15 min after administration (from 34.6 ± 2.3 to 28.5 ± 2.7 movements/min). It was thus similar to the effect of a dose of 50 mg/kg e.w. (Sedláček and Corner 1980). In 13-day-old embryos, in all three types of experiments, NAD significantly weakened the depressive effects of MPTP (Fig. 4, top part). In 17-day-old embryos it was virtually ineffective if administered together or after MPTP; if NAD was administered before MPTP, however, depression of motility was more pronouced than after MPTP alone (Fig. 4, bottom part).

The second part of the study concerned changes in the development of spontaneous motility after the chronic continuous administration of MPTP (3.57 mg/kg e.w./24 h) (Fig. 5). In 17-day-old embryos, without exception, the chronic administration of MPTP was manifested in a decrease of spontaneous motor activity. In series A – given MPTP from the 4th to 8th day of incubation – the effect of MPTP was not yet significant, but in the other groups it was already highly significant, with reduction of the frequency of spontaneous movements to only half the control value.

The administration of MPTP from the 8th to 12th day of incubation showed that the phase between the 4th and the 8th day was not decisive for the resultant effect and that the phase from the 12th to the 16th day did not intensify it any further. The chronic effect of MPTP was thus concentrated in the period between the 8th and the 12th day of incubation.

Discussion

The neurotoxic effect of MPTP is based on the damage it has on the dopaminergic mechanism, particularly in the nigrostriatal system (Heikkilla and Sonsalla 1987). In chick embryos, however, it was found that MPTP, on acute administration, depressed the motility not only in normal embryos with a complete CNS, but also in spinal preparations. It can thus be assumed that a target mechanism also exists in the spinal cord and that it ought to be a DA-ergic mechanism.This is borne out by the demonstrated sensitivity of spontaneous motility to the acute administration of DA in both normal and spinal chick embryos (Sedláček 1977).

Another aspect of all the effects of MPTP described in this study is that they were recorded during the embryonic period, i.e. not in primary correlation to a more advanced postnatal age of the brain tissue (Irwin *et al.* 1988). It is nevertheless impossible not to see the developmental differences in the late consequences of the acute administration of MPTP, in particular an insufficiency of the motor activity of hatching, which is associated with brain stem regulation of this motor act at least as far as the mesencephalon is concerned (Bakhuis and Corner 1973).

The general neurotoxic effect of MPTP can be evaluated according to the result of its acute administration with a lethal outcome in 11-day-old embryos and from the result of its continuous administration between the 8th and 12th day of incubation. Acute and long-term developmental depression of spontaneous motor activity and a marked association with supraspinal compartments of the brain are evidently special effects. Comparison is difficult, partly owing to a lack of comparative data on both the acute and the chronic effects of MPTP in different species (Markey and Schmuff 1986,Tab.1 and 2) and partly because of the dearth of basic information on developmental aspects of the effects of MPTP. Remarkable in this connection is the recovery of motility 24 h after the administration of MPTP in 13- and 17-day-old embryos and the significant weakening of recovery in 19-day-old embryos, which might be a manifestation of the similar correlation observed in mice (Saitoh *et al.* 1987).

The hypothetical mechanism of the neurotoxicity of MPTP associated with the neuroglial cells, whose monoaminooxidase B (MAO-B) oxidizes MPTP to $MPDP^+$, which is oxidized nonenzymatically to MPP^+. This ion is then taken up by DA-ergic endings, where the actual neurotoxic effect takes place (Javitch *et al.* 1985, Johannssen *et al.* 1985, Kopin and Markey 1988).

In experiments testing a block of MAO activity, the administration of MPTP after nialamide was found to have developmentally contradictory consequences; spontaneous motility was activated in 13-day-old embryos and the depressive effect of MPTP was potentiated in 17-day-old embryos. The effect in younger embryos corresponds in general to the biochemical scheme of the effect of MPTP as cited above. In that case, one could speculate that the mechanism of the effect of MPTP is more mature in the spinal cord of 13-day-old embryos and is immature in the supraspinal compartments of the CNS of 17-day-old embryos. Such developmental disparity in the effect of MPTP is evidently not new, as testified, for example, by the contradictory effect of prenatally administered MPTP on the postnatal development of tyrosine hydroxylase activity (Ochi *et al.* 1991).

In conclusion, therefore, it can be stated that 1. the tissue of the embryonic CNS is already sensitive to the neurotoxic effect of MPTP, 2. the effect, in this phase of development, is not bound only to strictly limited structures of the CNS, and 3. sensitivity and the image of the acute and chronic effect of MPTP are differentiated during the embryogenesis and maturation of the CNS.

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